

CLINICAL TRIALS

Pharmacokinetics and pharmacodynamics of PF-05190457: The first oral ghrelin receptor inverse agonist to be profiled in healthy subjects

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Received 9 February 2016; **Revised** 25 August 2016; **Accepted** 8 September 2016

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Keywords clinical trial, gastric emptying, ghrelin, growth hormone, Phase I, somnolence

AIM

To evaluate safety, tolerability and pharmacokinetics of oral PF-05190457, an oral ghrelin receptor inverse agonist, in healthy adults.

METHODS

Single (SAD) and multiple ascending dose (MAD) studies were randomised, placebo-controlled, double-blind studies. Thirty-five healthy men (age 38.2 ± 10.4 years; body mass index 24.8 ± 3.1 kg m⁻² [mean \pm standard deviation]) received ≥ 1 dose (2, 10, 40 [divided], 50, 100, 150, and 300 [single or divided] mg) of PF-05190457 and/or placebo in the SAD. In the MAD study, 35 healthy men (age 39.7 ± 10.1 years; body mass index 25.9 ± 3.3 kg m⁻²) received ≥ 1 dose (2, 10, 40 and 100 mg twice daily) of PF-05190457 and/or placebo daily for 2 weeks.

RESULTS

PF-05190457 absorption was rapid with a T_{max} of 0.5–3 hours and a half-life between 8.2–9.8 hours. PF-05190457 dose-dependently blocked ghrelin (1 pmol kg⁻¹ min⁻¹)-induced growth hormone (GH) release with (mean [90% confidence interval]) 77% [63–85%] inhibition at 100 mg. PF-05190457 (150 mg) delayed gastric emptying lag time by 30% [7–58%] and half emptying time by 20% [7–35%] with a corresponding decrease in postprandial glucose by 9 mg dL⁻¹. The most frequent adverse event reported by 30 subjects at doses ≥ 50 mg was somnolence. PF-05190457 plasma concentrations also increased heart rate up to 13.4 [4.8–58.2] beats min⁻¹ and, similar to the effect on glucose and ghrelin-induced GH, was lost within 2 weeks.

CONCLUSIONS

PF-05190457 is a well-tolerated first-in-class ghrelin receptor inverse agonist with acceptable pharmacokinetics for oral daily dosing. Blocking ghrelin receptors inhibits ghrelin-induced GH, and increases heart rate, effects that underwent tachyphylaxis with chronic dosing. PF-05190457 has the potential to treat centrally-acting disorders such as insomnia.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- PF-05190457 is a selective inverse agonist of the ghrelin receptor with a nonclinical profile supporting human dosing.
- Endogenous ghrelin and exogenous infusions in healthy subjects release growth hormone, stimulate hunger and increase gastric motility

WHAT THIS STUDY ADDS

- PF-05190457 is the first reported ghrelin receptor inverse agonist profiled in humans. It is safe and well-tolerated in healthy subjects with pharmacokinetics supporting daily oral dosing.
- PF-05190457 blocks ghrelin receptors in healthy subjects, dose-dependently increases heart rate, delays gastric emptying, induces somnolence and maximal inhibition for 2 weeks yields tachyphylaxis.

Tables of Links

| TARGETS |
|--|
| G protein-coupled receptors [2] |
| Ghrelin receptor |

| LIGANDS | |
|-----------------------------|---------------------------------|
| Ghrelin | Growth hormone 1 |
| Insulin | Pancreatic polypeptide |
| (–)-adrenaline | (–)-noradrenaline |
| Dopamine | C-peptide |
| Glucagon | Insulin-related growth factor 1 |
| Cortisol | Proatrial natriuretic peptide |
| Parathyroid hormone | T ₄ |
| Thyroid-stimulating hormone | |

These tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [1], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [2].

Introduction

Ghrelin is an endogenous 28-amino acid acylated peptide synthesised within the stomach fundus associated with modulating growth hormone secretion, gastric motility, gastric acid secretion and hunger. Acylated ghrelin is the only peripheral hunger-stimulating hormone that elevates preprandially in plasma, suggesting a role in meal initiation in humans [3]. Circulating endogenous baseline and pulsatile patterns of total ghrelin are inhibited in obese subjects following gastric bypass surgery [4, 5]; however, not all studies have been able to replicate a suppressant effect of Roux-en-Y gastric bypass on ghrelin [6, 7]. Endogenous levels of acylated ghrelin are reported to be decreased with increased body mass index (BMI) [8–10], elevated in obese type 2 diabetics [11] and inversely correlated with insulin sensitivity [12, 13]. Conflicting data on the association of endogenous ghrelin levels may be attributed to differences in patient populations used, assays or plasma sample handling [14, 15]. Exogenous administration of acylated ghrelin increases blood glucose and decreases insulin levels in both rodents and humans [16–23], and inhibits glucose-stimulated insulin secretion [24–26] and peripheral insulin sensitivity [27–32].

Acylated ghrelin is the endogenous ligand for the growth hormone secretagogue receptor 1a (ghrelin receptor [33]) of which des-acylated ghrelin has no activity. The metabolic effects of ghrelin were originally considered to be acting

centrally as ghrelin has been reported to cross the blood brain barrier [34]. However, more recently it has been shown that the direct effects of ghrelin on food intake are blocked by vagotomy in rodents and humans [35, 36] and the brain-impaired selective ghrelin receptor agonist capromorelin caused insulin resistance in humans [37]. Ghrelin receptors are located on islets and vagal afferent soma [38–49]. Ghrelin-induced food intake is blocked by vagotomy in rodents and humans [35] and ghrelin (intravenous) suppresses vagal afferent firing [35, 50–52]. Selective blockade of the gastric vagus nerve either by capsaicin (afferent only) or surgical differentiation (afferent and efferent pathways) blocks c-Fos expression within the arcuate nucleus in response to ghrelin-induced feeding. Vagotomy also eliminates ghrelin-induced growth hormone secretion indicating a novel vagus-mediated growth hormone secretion pathway [35], in addition to the known vagus-mediated efferent regulation of gastrointestinal motility, gastric acid secretion and nausea [53–55].

Despite a basic understanding of the physiology of ghrelin receptor activation in humans, the effect of blocking ghrelin receptors centrally or peripherally in humans as a potential pharmacotherapy for obesity and diabetes has been hampered over a decade by the inability to develop a safe effective ghrelin receptor antagonist to evaluate in humans. The ghrelin receptor is unique in that it has been shown to be constitutively active suggesting that blocking ghrelin receptor tone rather than blocking endogenous acylated

ghrelin binding may also be beneficial [56]. We have recently developed a ghrelin receptor inverse agonist, PF-05190457, that inhibits the constitutive activity of receptor in addition to competitively blocking activation by acylated ghrelin [57]. Here we report the pharmacokinetics (PK) and toleration profile of PF-05190457, which is the first ghrelin receptor inverse agonist to be profiled in humans.

Methods

Study design

Two clinical studies investigating characteristics of PF-05190457 in healthy volunteers are reported. Studies were conducted in compliance with the ethical principles originating in or derived from the Declaration of Helsinki, all International Conference on Harmonisation Good Clinical Practice Guidelines, and all local regulatory requirements (in particular, those affording greater protection to the safety of trial participants). Final study protocols and informed consent documents were reviewed and approved by the participating research centres and by an Independent Ethical Committee or Institutional Review Board. The principal investigator was required to inform the Independent Ethical Committee or Institutional Review Board of the study's progress and any serious and/or unexpected adverse events (AEs). A signed and dated informed consent was required before screening procedures were initiated.

Both studies were randomised, placebo controlled, double-blind and sponsor-open with oral doses of PF-05190457 [57]. The primary purpose of both studies was to evaluate safety and tolerability. The secondary purpose was to evaluate PK of PF-05190457, and tertiary objectives were to evaluate pharmacodynamics (PD) following oral PF-05190457 dosing. Nonstudy medications, caffeine, grapefruit juice (and related fruit juices), alcohol, nicotine, and exercise were restricted during study participation. Safety monitoring consisted of physical examinations, AE monitoring, 12-lead electrocardiograms, continuous cardiac monitoring over the first 8 hours after dosing (single ascending dose [SAD] study) or 2 hours prior to until 4 hours after dosing (multiple ascending dose [MAD] study), and vital sign and clinical safety laboratory measurements. For heart rate variability, two 20-min segments extracted at baseline and 2.5 h after dosing were sampled at 250 Hz from the continuous cardiac monitoring. Between 7 and 10 days after the last dose of treatment, subjects were requested to return for a follow-up assessment. Study medication was administered as an extemporaneously prepared suspension for all doses of active and placebo treatment in all cohorts of both studies. The study designs are graphically summarised in Figure S1, and a summary of the schedule of activities for both studies is presented in Table S1.

In both studies, meals were standardised by macronutrient content. While confined, the total daily nutritional composition was approximately 50% carbohydrate, 35% fat and 15% protein, and the daily caloric intake per subject did not exceed approximately 3200 kcal. In the SAD study, all meals were identical between cohorts and periods with the exceptions of the breakfasts on Day 1 for fasting (no breakfast),

fed (a high fat breakfast), and gastric emptying tests (breath test meal consisting of an [^{13}C] octanoic acid-containing omelette with a slice of white bread, as described below). In the MAD study, meals were identical for comparison of baseline and treatment days. The breakfast and lunch meals on Days -1, 1 and 14 did not include low pH food or drink including fruit juices and carbonated beverages, and these meals did not have high protein content (<30% protein). The subjects were monitored to consume approximately the same amount of each meal in a day and changes in meal consumption were estimated by the site (e.g. 80% of meal was consumed).

First-in-human, SAD study (NCT01247896)

The first-in-human study included single and divided doses of PF-05190457 or placebo. The ethics committee reviewing this study and associated documents was Comité d'Ethique de l'Hôpital Erasme (Brussels, Belgium). The study was conducted in a single centre where four cohorts of healthy volunteers completed the following parts of the study: Cohorts 1 and 2 (nine subjects each) participated in the dose-escalation part following a three-sequence crossover setting with placebo substitution; Cohort 3 (eight subjects) underwent a standard two-sequence crossover investigating gastric emptying; and Cohort 4 (nine subjects) underwent a two-period, three-sequence incomplete block with three divided-dose treatments.

Single, oral doses of PF-05190457 2 mg, 10 mg, 50 mg, 100 mg, 300 mg and 100 mg (fed) or placebo were administered in the dose escalation portion of the SAD study, Cohorts 1 and 2. In Cohort 3, subjects received a single PF-05190457 150 mg dose or placebo, and in Cohort 4, subjects received 40 mg or 300 mg as divided dose regimens (with breakfast, 2 h postbreakfast, with dinner, and 2 h postdinner).

Glucose was measured via glucometers and simultaneously in plasma. During dose-escalation, exploratory biomarkers were measured including acylated and total ghrelin, pancreatic polypeptide, adrenaline, noradrenaline, and dopamine. In Cohort 3, gastric emptying time of solid food was estimated by the stable isotope breath test [58]. This methodology is based on the gradually emptying of solid food (labelled with ^{13}C -octanoic acid) from the pylorus to the duodenum, where ^{13}C -octanoic acid is absorbed, metabolised in the liver to $^{13}\text{CO}_2$ and exhaled via the lungs. The gastric half-emptying time, the duration of the lag phase and the gastric emptying coefficient are continuously calculated by the analytical BreathID system from the $^{13}\text{CO}_2$: $^{12}\text{CO}_2$ ratios of the subject's exhaled air.

MAD study (NCT01372163)

The MAD study included 14-day, twice daily (BID) doses of PF-05190457 or matched placebo (with only a single dose on day 14). The institutional review board reviewing this study and associated documents was Integreview (Austin, Texas, USA). To characterise somnolence observed in the SAD further, three tasks from the CogState battery [59] (detection, identification, and one card learning) and Karolinska Sleepiness Scale was utilised [60] (data not shown).

In the MAD study, subjects received PF-05190457 at 2 mg, 10 mg, 40 mg or 100 mg doses BID or placebo.

Four primary biomarkers were selected for investigation in this study: the growth hormone (GH) response to an intravenous acylated ghrelin infusion, cortisol during the infusion, insulin during and separately from the infusion, and glucose during and separately from the infusion. Acylated ghrelin was infused for 180 min at approximately 2:30 PM (6.5 h post-dose) on Days -1, 1, and 14. The initial dose of acylated ghrelin was 5 pmol kg⁻¹ min⁻¹ [61] for 180 min, and this was reduced to 1 pmol kg⁻¹ min⁻¹ in Cohort 2 and subsequent cohorts to approximate the dose causing a half-maximal change in GH release (ED₅₀). Acylated ghrelin doses were calculated using the observed body weight at screening. Salivary cortisol was measured at time-matched points to examine graphically the relationship between PF-05190457 and cortisol by PF-05190457 dose with and without acylated ghrelin infusion (see Table S1 for measurement schedules). Fasting insulin and glucose were measured at baseline and on Days -1, 0, 1, 13, and 14, and the homeostatic model assessment (HOMA-B and HOMA-IR) were calculated to assess potential changes in beta-cell function and insulin sensitivity [62].

Subject selection

The inclusion criteria for both studies required healthy women of nonchildbearing potential or men aged 18–55 years, with a BMI between 17.5 and 30.5 kg m⁻², and weight between 50 and 100 kg with no clinically relevant abnormalities identified in medical history, physical examinations, vital signs, electrocardiograms or clinical laboratory tests.

Bioanalytical methods

Human plasma PF-05190457 samples obtained during these studies were analysed by the Sponsor (Pfizer Inc., Groton, CT, USA) using a validated liquid chromatography tandem mass spectrometric method within the assay stability and performance limits. The lower limit of quantification for PF-05190457 in plasma was 1.00 ng mL⁻¹ and the upper limit was 1000 ng mL⁻¹; samples above the upper limit of quantification were diluted into the calibration range. The inter-run coefficient of variation (CV) of the PF-05190457 assay at the lower limit of quantification was 12.5%. PK analyses were conducted on each subject who received PF-05190457 in the current study period. PK parameters were estimated using standard noncompartmental methods with the sponsor's internal software (eNCA) using actual PK sample collection times. The PK samples collected within ±10% of the scheduled postdose times were included in the mean plots, and all samples were included in the computation of noncompartmental parameters. Summary statistics were only calculated when ≥50% of subjects reported quantifiable or calculable parameters for the given parameter. Plasma concentrations below the quantification limit were assigned a value of 0.

Human plasma acylated ghrelin samples obtained during the MAD study were analysed by the Sponsor (Pfizer Inc., Groton, CT, USA) using validated liquid chromatography tandem mass spectrometric as described by Blatnik and Soderstrom [13] and Blatnik *et al.* [15].

Human serum anti-acylated ghrelin antibody samples obtained during the MAD study were stored at -20°C and subsequently analysed by QPS, LLC (Newark, DE, USA) using a validated enzyme-linked immunosorbent assay. A negative control of human serum and a positive control of rabbit polyclonal antibody to ghrelin (Abcam, Inc, Cambridge, MA, USA) were used within the assay. The assay cut-point was 1.41-fold the plate-mean negative control response while the positive control had a titre between 3.01 and 3.97-fold. The assay was staged with a screening assay; samples found positive with the screening assay were further tested with a confirmatory assay, and confirmed samples were titrated.

In the SAD study, subjects had a washout of at least 7 days between doses. Plasma PF-05190457 PK samples were collected at 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36 and 48 h postdose. Plasma PF-05190457 were collected at 0 (predose), 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 h postdose. In the MAD study, plasma PF-05190457 PK samples were collected on Day 1 at 0 (predose), 0.25, 0.5, 1, 2, 3, 6, 9.5 and 12 h postdose; predose on Days 2, 4, 6, and 9; on Day 13 at 0 (predose), 0.25, 0.5, 1, 2, 3, 6, 9 and 12 h postdose; and on Day 14 at 0 (predose), 1, 6, 9.5, 24 and 48 h postdose. Urine PF-05190457 PK samples were collected on Day 13 with the intervals of 0–4, 4–12 and 12–24 h postdose. Nonstandard plasma PK sampling schedule accommodated assessment of PF-05190457 PK and acylated ghrelin infusions.

Statistical analysis

The GH area under the concentration–time curve during the acyl ghrelin infusion (from 6 to 9.5 h [AUC_{6–9.5}]) was calculated on Days -1, 1 and 14 using linear trapezoidal method. Natural log transformed GH AUC_{6–9.5} were analysed using a mixed effects model with treatment, day and treatment by day as fixed effects, and subject within treatment as a random effect. Individual acylated ghrelin infusion rate was introduced in the model as a covariate; observed acylated ghrelin plasma concentrations were not used as a covariate in the model as they did not vary significantly within a dose group. Estimates of the adjusted mean differences between each active dose and placebo, and corresponding 90% confidence interval (CI) were obtained from the model for each dose and day separately.

For the gastric emptying crossover design, natural-log transformed gastric half-emptying time and lag time were analysed with mixed model analysis of variance. Treatment, sequence and period were fixed factors and subject was random factor nested within sequence.

Heart rate changes were modelled using a three-parameter Emax nonlinear mixed-effects model with each subject having a random effect on baseline and a fixed effects per nominal time of day to account for study-level and circadian variability as described by Conrado *et al.* [63] Uncertainty in the model parameters was assessed by 1000 nonparametric bootstrapping iterations.

To assess heart rate variability, telemetry data was analyzed by iCardiac (Rochester, NY, usa) using the spectral analysis outlined in the guidelines of the European Society of Cardiology [64]. The tachogram was computed for the original data after removal of ectopic beats. The frequency spectrum was computed using a fast Fourier transform, and total

power measured in the following frequency bands: very low frequency (0.0–0.04 Hz), low frequency (LF, 0.04–0.15 Hz), and high-frequency (HF, 0.15–0.40 Hz). The standard deviation of normal RR intervals, square root of the mean of the sum of the squares of differences between adjacent RR intervals, HF and low frequency were analysed. No formal statistical analysis was performed on these parameters considering the amount of data available at the end of the study.

Results

Subject disposition

In the SAD study, 35 healthy male subjects (38.2 ± 10.4 years; BMI: $24.8 \pm 3.1 \text{ kg m}^{-2}$) were randomised and received at least one dose of PF-05190457 and/or placebo, and no subject discontinued the study. In the MAD study, 35 healthy male subjects were randomised (39.7 ± 10.1 years; BMI: $25.9 \pm 3.3 \text{ kg m}^{-2}$; mean \pm standard deviation) and received at least one dose of PF-05190457 or placebo. One subject randomised to 100 mg BID discontinued due to non-treatment related AEs (see Safety section), and one subject discontinued for personal reasons prior to receiving PF-05190457 or placebo. The study was terminated by the sponsor after the fourth cohort (100 mg BID) due to tachyphylaxis of pharmacological activity. Study discontinuation was not a result of safety findings.

Safety

Safety analysis combined for both studies identified one serious AE (rhabdomyolysis) in the MAD study that had occurred post treatment and was not considered treatment-related. All other AEs were reported as mild or moderate in intensity, and no severe AEs occurred in either study. In both studies, there were also no temporary discontinuations or dose reductions due to AEs. In the MAD study, one subject treated with the 100 mg dose was discontinued permanently due to a mild face oedema, which developed on Day 5 of treatment and was considered not related to treatment but rather caused by a dental abscess.

The most common treatment-related AE was somnolence reported by 25 subjects at PF-05190457 doses ≥ 50 mg in the SAD study. The somnolence AEs were considered treatment-related, and the proportion of subjects reporting somnolence appeared to be related to PF-05190457 exposure (Table 1). In the MAD study, somnolence was observed in five subjects treated with 100 mg BID doses of PF-05190457 and in three subjects in the placebo group. In general across both studies, the events of somnolence were mild and began at approximately T_{\max} resolving 6–9 hours later. There were no notable changes in Karolinska Sleepiness Scale or CogState tests with administration of PF-05190457 in the MAD study.

Changes in pulse rate were observed in both studies primarily in the time shortly after dosing as described by a concentration-responsive PK/PD model. In the SAD study, a treatment-related increase from baseline was observed, which was associated with the level of PF-05190457 exposure (Figure 1A). In the MAD study, the mean time-matched change from baseline supine pulse rate on Day 1 indicated a concentration-responsive increase up to 13.4 [4.8–58.2]

Table 1

Number of subjects with an event of somnolence in single dose and multiple dose studies

| Single ascending dose study NCT01247896 | Placebo(n = 32) | 2 mg(n = 6) | 10 mg(n = 6) | 40 mg Divided(n = 6) | 50 mg(n = 6) | 100 mg Fed(n = 9) | 150 mg(n = 8) | 300 mg P2(n = 6) | 300 mg P3(n = 6) | 300 mg Divided(n = 6) |
|---|-----------------|-----------------|------------------|----------------------|-------------------|-------------------|---------------|------------------|------------------|-----------------------|
| Somnolence | 3 | 1 | 1 | 1 | 3 | 7 | 5 | 3 | 6 | 1 |
| Multiple ascending dose study NCT01372163 | Placebo(n = 11) | 2 mg BID(n = 6) | 10 mg BID(n = 6) | 40 mg BID(n = 5) | 100 mg BID(n = 6) | | | | | |
| Somnolence | 3 | 0 | 0 | 0 | 5 | | | | | |

BID, twice a day

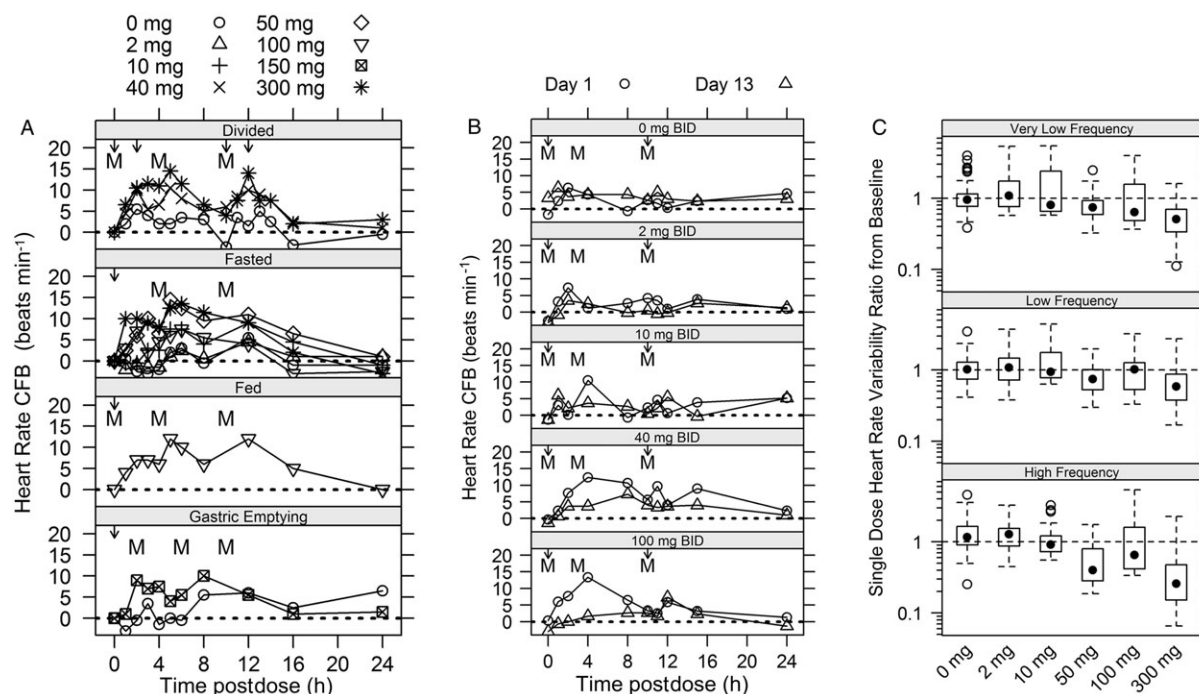


Figure 1

Heart rate. (A) Single ascending dose (SAD), (B) multiple ascending dose (MAD) showing tachyphylaxis and (C) clinical heart rate variability (HRV). (A) SAD study median heart rate change from baseline (CFB) with lines indicating PF-05190457 doses (0–300 mg) and panels for divided dose administration (dosed with breakfast, 2 h post breakfast, with dinner, and 2 h post dinner; Cohort 4), fasted (dosed without breakfast; Cohorts 1 and 2), fed (dosed with breakfast; Cohort 2) and gastric emptying examination (dosed 2 h before a stable isotope gastric emptying test meal; Cohort 3). “M” indicates the time of a meal, and arrows from the top of the panel indicate the time of dosing. (B) MAD study heart rate tachyphylaxis. Lines compare the heart rate change from time-matched baseline (CFB) to Day 1 or 13; panels indicate increasing dose of twice-daily (BID) PF-05190457. “M” indicates the time of a meal, and arrows from the top of the panel indicate the time of dosing. (C) HRV by increasing PF-05190457 dose in the fasted state during the SAD study Cohorts 1 and 2 with panels for HRV power in a frequency band. Boxes represent the 25th to 75th percentiles of observations; whiskers represent the farthest observation ≤ 1.5 -times the inter-quartile range from the top or bottom quartile; closed circles are the median for the treatment and open circles are observations outside the whiskers

beats min^{-1} with apparent tachyphylaxis by Day 14 (Figure 1 B). Together, the concentration-related heart rate increases and episodes of somnolence determined that the 300 mg dose of PF-05190457 was the maximum tolerated dose in the SAD study. Heart rate variability (HRV) analysis from the SAD study suggested a potential dose-responsive decrease in high frequency power, with a lesser decrease in low frequency power; very low frequency power appeared not to change dose-responsively (Figure 1C).

In both the SAD and MAD studies, the incidence of laboratory abnormalities did not suggest a trend of a treatment-related effect. In the MAD study, three subjects had anti-acylated ghrelin antibodies above the limit of quantification during the study. One subject (a 53-year-old male in the placebo group) entered with a titre below the limit of quantification (BLQ, <50 arbitrary units [U]) and had measurable titres on Day 16 (74 U) and Day 21 (80 U); by Day 42, the titre had returned to BLQ. The second subject (a 50-year-old male in the 2 mg BID group) entered the study with a BLQ titre and had measurable titres on Days 16 (332 U), 21 (372 U) and 42 (354 U). The third subject had an elevated titre of 60 U at baseline, showed an increase on Day 16 to 103 U and then demonstrated titres at a plateau at 107–110 U; the predose antibodies observed are potentially

due to autoantibody formation against the native protein. None of the subjects reported AEs that appeared related to the increase in the antibody titre.

In the SAD and MAD studies, troponin-I and proatrial natriuretic peptide (pro-ANP) were measured to ensure cardiac safety, and neither marker showed any adverse changes. In the MAD study, biomarkers for bone health (calcium, ionised calcium, osteocalcin, parathyroid hormone and serum bone-specific alkaline phosphatase) and thyroid function (free T_4 and thyroid-stimulating hormone) were measured and none showed any adverse changes.

Pharmacokinetic evaluations

Plasma PK concentrations are shown in Figures S2 and 2 for the SAD and MAD studies respectively, and noncompartmental parameters for the studies are in Tables 2 and S3. Absorption was rapid across all doses with median T_{max} between 0.5 and 3.0 h. Increases in both AUC and maximum observed plasma concentration (C_{max}) were supraproportional from 2 to 50 mg and approximately dose-proportional from 50 to 300 mg. The arithmetic mean half-life was between 8.2 and 9.8 h when concentrations were

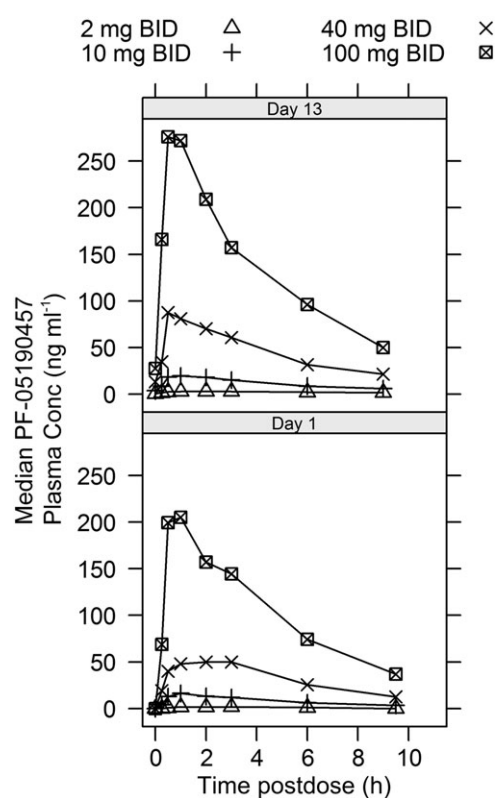


Figure 2

Multiple ascending dose study median PF-05190457 plasma concentrations

above the limit of quantification for the duration of measurement; shorter half-lives were probably due to limited time above the limit of quantification. With BID dosing,

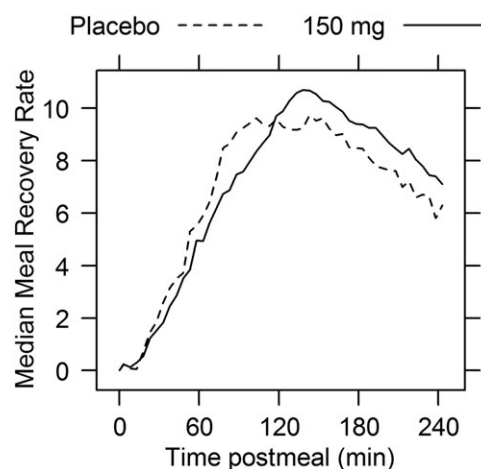


Figure 3

Median gastric emptying rate of isotope recovery by time with 150 mg PF-05190457 or placebo from the single ascending dose study Cohort 3

accumulation ratios for AUC_{τ} were approximately 1.3-fold and steady-state was achieved by Day 2 as predicted from the SAD study. As expected from the dose proportionality, divided dosing lowered C_{max} , increased T_{max} , and did not affect AUC. Renal elimination of PF-05190457 was <3% of the dose in all doses tested in the MAD study.

Pharmacodynamic evaluations

In the SAD study Cohort 3, single dose administration of 150 mg PF-05190457 compared to placebo in the same subjects delayed the gastric emptying lag time by 30% [7–58%] (least squares mean [90% CI]) and the half-emptying time by 20% [7–35%] without significantly affecting the

Table 2

Summary clinical pharmacokinetic parameters from the multiple ascending dose study

| PF-05190457 Dose/regimen | Study day | N | $AUC_{0-\tau}$ (ng·hr ml ⁻¹) | C_{max} (ng ml ⁻¹) | T_{max} (hr) | Urinary Ae% |
|--------------------------|-----------|---|--|----------------------------------|-------------------|-------------|
| 2 mg BID, fed | 1 | 6 | 10.8 (39) | 2.3 (34) | 1.00 (0.50, 3.02) | |
| | 13 | 6 | 22.2 (17) | 3.2 (20) | 2.00 (1.00, 3.00) | 1.6 (21) |
| | 14 | 6 | 21.1 (21) | 3.5 (26) | 1.00 (0.98, 6.00) | |
| 10 mg BID, fed | 1 | 6 | 79.9 (24) | 16.1 (32) | 1.05 (0.50, 3.00) | |
| | 13 | 6 | 112.3 (15) | 21.2 (18) | 1.00 (0.50, 2.00) | 2.0 (33) |
| | 14 | 6 | 109.2 (21) | 21.9 (37) | 1.00 (1.00, 1.00) | |
| 40 mg BID, fed | 1 | 5 | 334.7 (31) | 56.3 (35) | 3.00 (0.50, 3.07) | |
| | 13 | 5 | 461.8 (24) | 83.4 (16) | 0.52 (0.50, 1.00) | 2.2 (17) |
| | 14 | 5 | 434.9 (28) | 86.2 (26) | 1.00 (1.00, 2.40) | |
| 100 mg BID, fed | 1 | 6 | 985.0 (23) | 201.9 (34) | 1.25 (0.50, 2.00) | |
| | 13 | 5 | 1285 (23) | 273.1 (31) | 0.50 (0.50, 1.00) | 2.7 (20) |
| | 14 | 5 | 1235 (23) | 263.1 (42) | 1.00 (1.00, 1.00) | |

$AUC_{0-\tau}$, C_{max} , Urinary Ae: Geometric mean (%CV); T_{max} : median (range)

Ae%, amount excreted as percent of dose; AUC, area under the plasma concentration–time curve; BID, twice a day; C_{max} , maximum observed plasma concentration; %CV, coefficient of variation; T_{max} , time of C_{max}

gastric emptying coefficient (Figure 3). In the same study, average postlunch glucose ($AUC_{4-7/3}$) appeared to be decreased by up to 9 mg dL^{-1} at the top single dose of 300 mg in the fasted state (Cohorts 1 and 2), and there was no notable change (Figure S3A) in acylated ghrelin (AUC_{0-6}), pancreatic polypeptide (AUC_{0-8}), adrenaline (AUC_{0-24}), noradrenaline (AUC_{0-24}), dopamine (AUC_{0-24}), insulin (AUC_{0-24} ; Figure S3C), C-peptide (AUC_{0-24}), or glucagon (AUC_{0-24}). Biomarker data from the SAD study are summarised in Table S4.

Following Cohort 1 in the MAD study, steady-state acylated ghrelin concentrations during the $5 \text{ pmol kg}^{-1} \text{ min}^{-1}$ 180-min infusion were determined to be approximately five-fold higher than the nonclinically-determined concentration yielding EC_{50} . Due to the assessment of higher acylated ghrelin concentrations, the acylated ghrelin infusion rate was lowered to $1 \text{ pmol kg}^{-1} \text{ min}^{-1}$ for 180 min for Cohorts 2–4. Ghrelin-induced GH release is shown by box and whisker plots for individual ratio of the change from baseline $AUC_{6-9.5}$ (Day 1/Day –1, Day 14/Day –1) are presented by dose and day in Figure 4 and ratios to baseline and to placebo are in Table 3. On Day 1, a PF-05190457 dose-responsive relationship of GH was observed relative to baseline in the same subject; tachyphylaxis appeared to be observed by Day 14 when no dose caused a significant reduction in GH release.

Weighted mean daily glucose plasma concentration assessed at baseline (Day 0) and steady-state (Day 13) were comparable (Figure S3 panel B). Additionally, insulin (AUC_{0-8} ; Figure S3 panel D), homeostatic model assessment for β -cell function and insulin resistance, acylated ghrelin levels during the ghrelin infusion ($AUC_{6-9.5}$), insulin-related growth factor 1 during the ghrelin infusion ($AUC_{6-9.5}$), salivary cortisol (AUC_{0-8}), and urine catecholamines did not appear to be affected by PF-05190457 administration (Table S5).

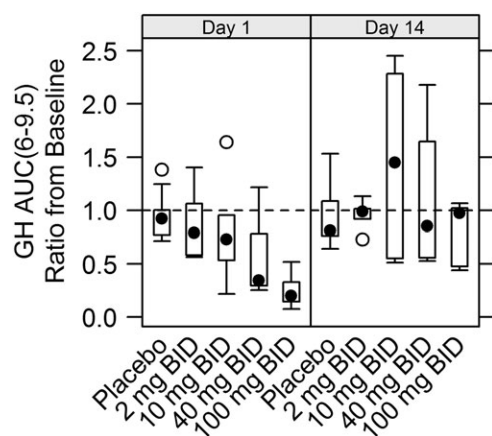


Figure 4

Ghrelin-induced growth hormone (GH) area under the concentration–time curve during the acyl ghrelin infusion (from 6 to 9.5 h: $AUC_{6-9.5}$) by treatment and day in the multiple ascending dose study. Note that Cohort 1 had $5 \text{ pmol kg}^{-1} \text{ min}^{-1}$ ghrelin infusion while Cohorts 2–4 had $1 \text{ pmol kg}^{-1} \text{ min}^{-1}$ ghrelin infusion. One subject in the placebo group had intravenous infiltration during infusion on Day 1 and was omitted from the figure

Discussion

Novel findings in this study include: (1) the first report of a ghrelin receptor inverse agonist profiled in humans; (2) validation of PF-05190457 as a safe and well-tolerated inhibitor of the ghrelin receptor with an acceptable PK profile for daily oral dosing; (3) acutely blocking the ghrelin receptor in healthy volunteers, dose-dependently increases heart rate, delays gastric emptying and induces somnolence; (4) chronically, tachyphylaxis occurs when peripheral ghrelin receptors are maximally inhibited; and (5) PF-05190457 may be suitable to assess central mechanisms where transient inhibition of the receptor is required.

Ghrelin receptor agonists have been extensively profiled in humans primarily focusing on frailty [37, 65], gastroparesis [66] and cachexia [67]. However, despite ghrelin and its receptor being discovered nearly 20 years ago, no ghrelin receptor antagonist had safely progressed to humans to facilitate our basic understanding of the role of endogenous ghrelin via the ghrelin receptor in obesity, diabetes, addiction, or psychiatric disorders [68]. Preclinically, PF-05190457 is a potent ghrelin receptor antagonist with inverse agonist activity and has been reported to increase calcium in dispersed rat islets, increase insulin secretion from human islets and increases rat vagal afferent firing *ex vivo* [57, 69]. PF-05190457 is the first ghrelin receptor inverse agonist to be profiled in humans.

PF-05190457 PK was well-behaved with low- to moderate-variability in AUC and C_{max} . MAD study PK was well-predicted from the single-dose PK in the SAD study; $AUC_{0-\tau}$ at steady-state was similar to AUC_{0-inf} in the SAD study. The increase in C_{max} and AUC with increasing dose appears to be supraproportional at doses $\leq 50 \text{ mg}$ with a change in dose proportionality $\geq 100 \text{ mg}$. The change in proportionality without significant changes in half-life suggests that it is related to absorption and not clearance.

Ghrelin is primarily known for its GH-releasing properties [70]. To confirm that PF-05190457 is pharmacologically blocking the ghrelin receptor, an ED_{50} of ghrelin was intravenously infused to subjects to stimulate GH [61]. The ghrelin infusion rate was selected to be near its ED_{50} so that 10-fold the inhibitory constant (K_i) of PF-05190457 would be expected to block the response by approximately 90%. The ghrelin ED_{50} was determined in this study to be lower than the literature-reported $5 \text{ pmol kg}^{-1} \text{ min}^{-1}$, and the ghrelin infusion rate of $1 \text{ pmol kg}^{-1} \text{ min}^{-1}$ was found to better approximate the ED_{50} . As expected, PF-05190457 dose-dependently inhibited the ghrelin-induced GH with a maximum inhibition observed at a 100 mg PF-05190457 BID, which achieved maximum free concentrations of approximately 18-fold the human K_i [57, 69].

Whilst the ghrelin receptor block peripherally was expected to be $>80\%$ for 24 hours, the expected block centrally was likely to be considerably less due to the brain impairment of PF-05190457 (Table S2). Based on the rat and dog brain/plasma ratio, approximately 70% human central receptor occupancy was achieved for ≥ 3 hours after each dose at 100 mg PF-05190457 BID. This was supported by the observation of transient somnolence at $\geq 50 \text{ mg}$ PF-05190457 (free concentrations seven-fold K_i at C_{max} and $>K_i$ for approximately 10 hours after single doses). The tachyphylaxis

Table 3

Ghrelin infusion-induced growth hormone release changes with PF-05190457 administration. Values are least squares means [90% CI]; ghrelin infusion rate was included as a covariate in the least squares mean model

| PF-05190457 Treatment | Growth hormone AUC _{6-9.5} ratios (unitless) | | | |
|--------------------------|--|-------------------|--------------------|-------------------|
| | Ratio from baseline | | Ratio from placebo | |
| | Day 1 | Day 14 | Day 1 | Day 14 |
| Placebo ^{a,b} | 0.91 [0.70, 1.20] | 0.92 [0.71, 1.19] | – | – |
| 2 mg BID ^a | 0.73 [0.40, 1.34] | 0.85 [0.47, 1.56] | 0.80 [0.41, 1.58] | 0.93 [0.47, 1.84] |
| 10 mg BID | 0.70 [0.47, 1.04] | 1.19 [0.80, 1.77] | 0.77 [0.48, 1.23] | 1.30 [0.82, 2.07] |
| 40 mg BID | 0.45 [0.28, 0.72] | 0.97 [0.61, 1.55] | 0.50 [0.29, 0.84] | 1.06 [0.63, 1.79] |
| 100 mg BID | 0.21 [0.14, 0.31] | 0.86 [0.57, 1.29] | 0.23 [0.15, 0.37] | 0.94 [0.58, 1.51] |

AUC, area under the curve; BID, twice a day

^aSubjects in cohort 1 were administered 5 pmol kg⁻¹ min⁻¹ (two placebo and all subject administered 2 mg BID) while all other subjects were administered 1 pmol kg⁻¹ min⁻¹

^bOne subject had an acylated ghrelin intravenous infiltration on Day 1 and was excluded from Day 1 calculations.

observed with chronic dosing on the ghrelin-induced GH and not on somnolence is further supportive that the tachyphylaxis was due to blocking the ghrelin receptor 24/7 systemically whereas transient blockage for a short period of time centrally daily may not lose efficacy.

Exogenous ghrelin increases blood glucose and decreases plasma insulin in humans and rodents [16–23], and preclinically the hyperglycaemia is abolished by the peptide ghrelin receptor antagonist [D-Lys3]-GHRP-6 [22]. Ghrelin infusion in rodents and humans also inhibits glucose-stimulated insulin secretion *in vivo* [25, 26, 40]. The inhibitory effects of ghrelin on insulin secretion *in vivo* are via the ghrelin receptor located on islets [35, 41, 46, 48]. *Ex vivo*, ghrelin decreases glucose-induced insulin release in rodent islets and perfused pancreas [22, 24, 25, 71, 72]. PF-05190457 increased intracellular calcium within rat dispersed islets and increased insulin secretion from human islets *ex vivo* in a glucose-dependent manner [57, 69]. However, PF-05190457 did not act as an insulin secretagogue in healthy volunteers as postprandial insulin was not modulated. It remains to be determined whether insulin would be modulated in patients with type 2 diabetes. In contrast, postprandial glucose was modestly reduced, potentially due to the delay in gastric emptying. With single PF-05190457 doses, gastric emptying rate and half-emptying time were decreased by 20–30%. These data support the role of endogenous ghrelin in gut motility. Ghrelin receptor agonists are currently under investigation for diabetic gastroparesis by Rhythm Pharmaceuticals (RM-131) and Tranzyme (TZP-102).

Ghrelin infusion studies in lean healthy volunteers have been reported to decrease blood pressure, an effect consistently reported in rodent studies attributed to an inhibition of sympathetic tone [73–77]. Acutely blocking ghrelin receptors with PF-05190457 in lean healthy subjects had no effect on blood pressure suggesting lack of endogenous tone or receptor constitutive activity. In contrast, blocking ghrelin receptors temporarily increased heart rate in the absence of observed changes in blood pressure. Despite a number of cardiovascular reports investigating ghrelin, there is a paucity of

data linking ghrelin with heart rate. Soeki *et al.* reported [76] a decrease in heart rate in healthy volunteers, which accommodated a decrease in blood pressure. The unexpected difference observed with PF-05190457 may be explained by direct effects at higher exposures blocking ghrelin receptors within the brain. In the anaesthetised rabbit, central administration of ghrelin solely increased heart rate via increased acetylcholine into the sinoatrial node following activation of efferent vagal nerves [76] suggesting that central ghrelin receptors modulate the parasympathetic nervous system. Observations of HRV analysis have shown that parasympathetic activity is a major contributor to the HF component [64]. Thus the dose-responsive decrease in high frequency power suggests a decrease in parasympathetic to sympathetic activity with PF-05190457 administration, which is opposite of the effect to a ghrelin infusion on HRV where sympathetic activity is decreased relative to parasympathetic [73, 76].

The tachyphylaxis observed with blocking the ghrelin receptor chronically on the ghrelin-induced GH surge (Figure 4 and Table 3), heart rate and post-prandial glucose lowering is probably attributed to: (1) desensitisation of the ghrelin receptor; or (2) the mechanism underlying efficacy. Camina *et al.* [78] observed that the ghrelin receptor desensitises upon stimulation and slowly recycles within 360 min. As PF-05190457 is an inverse agonist, it is possible that desensitisation occurs with binding, resulting in chronic exposure preventing membrane bound receptors being present for activation. However, receptor desensitisation is unlikely because after 2 weeks of PF-05190457 treatment, ghrelin reproducibly stimulate GH release. More plausible is the underlying ghrelin receptor-mediated vagal pathway became redundant. Ghrelin effects on heart rate, GH and glucose lowering have all been shown to be mediated via the vagus nerve as highlighted directly using vagotomy or indirectly via heart rate variability [73]. A similar comparison of vagal tachyphylaxis has been used to explain differences in efficacy with chronic dosing of injectable GLP-1 agonists [79–81]. Exendin, which has a short half-life requiring BID dosing, has a greater effect on postprandial glucose in part

via a vagally mediated delay in gastric emptying, whereas liraglutide once a day dosing has a greater effect on the fasting plasma glucose attributed to a direct effect on insulin secretion rather than on gastric emptying.

Ghrelin has been associated with multiple physiological and pathophysiological mechanisms within the central nervous system, namely sleep, reward, mood and memory [82]. The PK profile of PF-05190457 favours its use in centrally-mediated mechanisms. The increase in somnolence with higher doses of PF-05190457 and appeared initiate at T_{max} typically resolving 6–9 hours after symptoms started. Evidence supporting that the somnolence observation being centrally-mediated is: (1) increased somnolence with increasing doses and ultimate pharmacological exposures within the brain; and (2) in rodents intracerebroventricular and intrahypothalamic injections but not systemic ghrelin induces wakefulness and suppression of nonrapid eye movement (REM) and REM sleep, with impaired wake-promoting mechanisms in ghrelin receptor knock-out mice [83–86]. Studies related to peripheral ghrelin levels are conflicting with decreased endogenous levels of systemic ghrelin linked to insomnia [87] whereas exogenous ghrelin administration improves non-REM sleep [88–91].

The mechanism of action underlying central action of ghrelin in inducing wakefulness has not been fully defined but hypothesised to involve stimulation of orexin neurones. Orexin neurones, which reside in the lateral hypothalamus are well known for maintaining wakefulness [92]. Microinjection of ghrelin into the lateral hypothalamus has a strong wakefulness-promoting effect. The role of peripheral ghrelin has been hampered by lack of quantification of: (1) how much is accessing the brain to trigger wakefulness; and (2) plasma levels that modulate GH. Peripheral ghrelin levels are positively associated with GH [93]. The nocturnal burst of GH that occurs with NREM sleep seems to be critical in the maintenance in sleep–wake cycle and regulating fasting glucose levels [94, 95].

Together, preclinical studies and the current study highlight the role of central ghrelin in arousal and utility of PF-05190457 in sleep disorders. Further studies are required to evaluate the effect of PF-05190457 on T2D, weight loss in Prader–Willi patients, prevention of weight regain in subjects with associated high levels of acylated ghrelin, sleep quality and circadian metabolic disorders i.e. night eating syndrome, jet-lag or shift worker disorder.

Competing Interests

These studies were sponsored by Pfizer. All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: W.S.D., G.E.S., S.C.J., T.T. and V.M.J. had support from Pfizer for the submitted work; W.S.D., G.E.S., S.C.J., T.T. and V.M.J. were employees with Pfizer in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work. Following discontinuation of clinical development of PF-05190457 for the treatment of Type 2 diabetes, the molecule was provided to the United States National Institute of

Health for its National Center for Advancing Translational Sciences program. PF-05190457 is currently under investigation for treatment of alcoholism (ClinicalTrials.gov Identifier: NCT02039349).

The authors would like to acknowledge the following individuals who contributed to various parts of the studies: Jigna Patel for her efforts to generate and support PF-05190457 and acylated ghrelin formulations, Chandra Vage for her assistance with non-clinical pharmacodynamic assessments, Kimberly Lapham for her nonclinical development support, and the investigators and subjects in the studies for their participation.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

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Figure S1 Single ascending dose (SAD) and multiple ascending dose study designs. Each shaded box indicates one cohort of subjects. Arrows indicate washout between periods within a cohort. In each study, the cohorts were sequential in each study. In the SAD study, cohort 2 period 3 was a repeated dose (indicated by †). Each dose was placebo-controlled (either the indicated dose or placebo) unless denoted by ‡ to indicate no placebo in the given regimen. In the SAD study, cohort 4 had divided doses (denoted by ¶) with doses administered at 0 (with breakfast), 2, 10 (with dinner), and 12 hours relative to the first dose in the day. In the multiple ascending dose study, doses were administered twice daily (BID) for 14 days with only a single dose on Day 14

Figure S2 Single ascending dose pharmacokinetics with panels by treatment administration conditions and lines by treatment. Lines indicate PF-05190457 doses (2–300 mg) and panels for divided dose administration (dosed with breakfast, 2 h post breakfast, with dinner, and 2 h post dinner; Cohort 4), fasted (dosed without breakfast; Cohorts 1–3), and fed (dosed with breakfast; Cohort 2)

Figure S3 Mean glucose (panels A and B) and insulin (panels C and D) for the single ascending dose study (panel A and C) and the multiple ascending dose study (panel B and D). In the single ascending dose study, meal timing differed by cohort: Cohort 1 meals were at 4 and 10 h post dose; Cohort 2 at 0 (fed treatment), 4 and 10 h; Cohort 3 at 2 (gastric emptying meal), 6 and 10 h; and Cohort 4 at 0, 4 and 10 h. In the multiple ascending dose study, meals were at 0, 3 and 10 h on days 1 and 14. “M” indicates the time of a meal, and arrows from the top of the panel indicate the time of dosing

Table S1 Summary of schedule of activities

Table S2 Mean rat PF-05190457 pharmacokinetic parameters (including central exposure)

Table S3 Summary clinical pharmacokinetic parameters from the first human single-ascending dose study

Table S4 Summary clinical biomarkers in the single ascending dose (SAD) study

Table S5 Biomarkers prior to and during acyl ghrelin infusion. Note that three placebo subjects and all 2 mg twice daily subjects received 5 pmol kg^{−1} min^{−1} infusion while other subjects received 1 pmol kg^{−1} min^{−1}